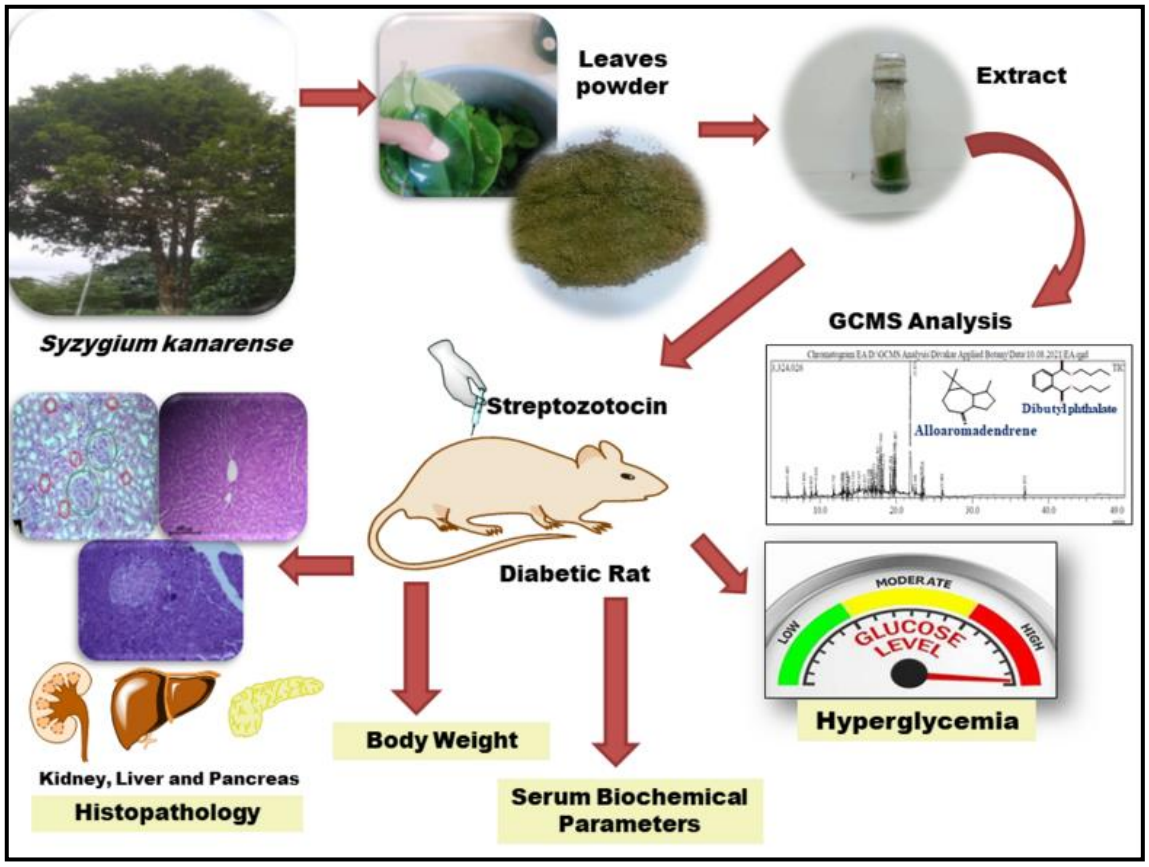
ABSTRACT

The genus *Syzygium* is widely used as a traditional medicine to treat diabetes. The phytochemicals, GC-MS analysis, alpha-amylase and alpha-glucosidase inhibition, toxicity, and free radical scavenging capacity of *Syzygium kanarense* leaf extracts for the diabetic condition were investigated. Streptozotocin was injected into male Wistar rats to induce diabetes. The rats were separated into two groups: untreated rats and diabetic-induced rats treated with extracts and glibenclamide respectively. After 21 days, blood parameters such as glucose levels, liver, kidney function and lipid profile were evaluated.

In a cytotoxicity study, the ethyl acetate and aqueous leaf extracts of *S. kanarense* indicated a proliferative effect on the cell line, with lesser toxicity compared to petroleum ether and hexane extracts. The GC-MS study revealed the existence of many phytochemicals in ethyl acetate and aqueous extracts. In the diabetic rat model, treated with *S. kanarense* leaf extracts of ethyl acetate and aqueous extracts reduced blood glucose levels with fewer side-effects compared to the reference drug glibenclamide. The results indicated that ethyl acetate and aqueous leaf extracts of *S. kanarense* possess substantial antidiabetic activity comparatively; the former was found to be more effective.

Graphical abstract



CHAPTER -1 INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Diabetes mellitus (DM) is a group of metabolic diseases that results in a high level of sugar in the blood due to defective insulin action or reduced insulin secretion (Patel *et al.,* 2012). So, it refers to a group of common complex metabolic disorders that are characterized by hyperglycemia (American Diabetes Association, 2009). DM and its complications are still expensive to control with modern drugs. The chronic type of this condition is connected with long-term effects and abnormal function of several organs such as the heart, liver, kidneys, and blood vessels. The extensive research for antidiabetic drugs over the past decade has established plant products as one of the most important sources for drug discovery. Natural products keep on playing a significant role in novel drugs in the pharmaceutical production (Mohanraj *et al.,* 2018; Newman and Cragg, 2020). The significance of natural products for multiple uses is attributed to their different biological activities, low toxicity, and ecological sustainability. There are several advantages of using plant products for health-related problems as the risk of death can be reduced, organ injury recovered and the occurrence of health problems can also be effectively decreased. Based on these attributes natural plant products are reported for the treatment of DM.

Syzygium is a genus of flowering plants, under the Myrtaceae family and is traditionally widespread in tropical and subtropical regions. Many *Syzygium* genera are used for antidiabetic activities. The antidiabetic activities of *Syzygium cumini* and *Syzygium aromaticum* are well documented (Chaudhry *et al.,* 2016; Lakshmi and Manasa, 2021). *Syzygium* is a tropical tree and numerous reports have shown it to be a valuable therapeutic agent. Among the *Syzygium* genus, *Syzygium kanarense* is an unexplored rare plant described by Talbot (1897) from the evergreen forests of Gerusoppa Ghat and was reported to be endemic to Shimoga and the north Kanara district of Karnataka, India (Ahmedullah and Nayar, 1986). However, the species was recollected after 67 years by R. Sundararaghavan but this was not reported even after the species was deposited at the BSI Western Circle in Poona. Later, in 1996, Nayar reported that *S. kanarense* was an endangered species last seen by Talbot discounting the R. Sundararaghavan collection. After a long period of 47 years, this plant was been rediscovered in evergreen forests (Shenoy *et al.,* 2015). The essential oil of *S. kanarense* reported 52 constituents from the aerial parts (Joshi *et al.,* 2017).

The phytochemicals had been shown to inhibit the activity of glucose-promoting enzymes such as alpha-amylase (Li *et al.,* 2009) and alpha-glucosidase (Xu, 2010). The most important influential factor in toxicity study is the dose selection of the herbal extracts. There is no specific dosage for the extracts but a dose that does not induce toxicological effects is recommended (Tiwari *et al.,* 2014). However, there are no reports of toxicological studies of the rare plant of *S. kanarense*. Therefore, this study was focused on *in vitro* (Cytotoxicity by MTT and Brine shrimp lethality assays) and *in vivo* toxicity (Acute oral toxicity) to ensure the safety of the leaf extracts for antidiabetic study. To study *in vitro* toxicity of extracts, the cytotoxicity test is the best biologically evaluated method that uses tissue cells to screen toxicity by cell growth (Li *et al.,* 2015). After a non-toxic effect was proved by an *in vivo* study in leaf extracts, the study continued to reveal its antidiabetic potential in the animal model of albino Wistar rats. The present study, the prolonged effect (up to 21 days) of the ethyl acetate and aqueous leaf extracts of *S. kanarense* in fasting blood glucose (FBG) level and biochemical parameters such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), serum glutamic pyruvic transaminase (SGPT), creatinine, total protein, urea, albumin, serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase were studied in streptozotocin (STZ) induced diabetic rats. Hence on the above fact, no study has been carried out on leaf extracts of *S. kanarense* in STZ-induced diabetic rats. Thus, the present study is an attempt to investigation the antidiabetic activity of *S. kanarense* leaf extracts.

1.2 Review of literature

This section gives the literature relevant to the present study. The chapter also deals with the fundamental and applied aspects of DM **(**Xie *et al.*, 2003; Bhat *et al.*, 2008), Types of DM (Colberg *et al.,* 2010; Maahs *et al.,* 2010), risk factors (Cukierman-Yaffe *et al.*, 2009), management of DM (Donga *et al.,* 2011), natural products, significance of alpha-amylase and alpha-glucosidase in diabetes (Barrett and Udani, 2011), Genus of *Syzygium* used for the management of diabetes (Konda *et al.,* 2019; Lakshmi Manasa, 2021), safety analysis of cytotoxicity, acute oral toxicity and the animal models utilized to test for antidiabetic activities in recent studies were reviewed. Furthermore, the experimental procedure was explained.

AIM AND OBJECTIVES

The aim of this study was to examine the *S. kanarense* leaf extracts for alpha-amylase and alpha-glucosidase inhibition activity and to determine the effect of the extracts in a streptozotocin (STZ) induced diabetic rat model.

Objectives

- To evaluate phytochemical constituents, total phenolics, antioxidant activity and GC-MS analysis of leaf extracts.
- To evaluate the safety of leaf extracts from toxicity in an *in vitro* and *in vivo* model.
- To evaluate the inhibition activity of leaf extracts on alpha-amylase and alpha-glucosidase.
- To evaluate the efficacy of the leaf extracts in diabetic rats comparing with diabetic, non-diabetic and standard drug-treated rats.

The whole work is divided into five-chapters.

The first chapter is an introduction and a literature review. The second chapter is the phytochemicals screening, antioxidant activity and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *S. kanarense* leaf extracts. Chapter three deals with the toxicity study on *S. kanarense* leaf extracts. The fourth chapter focuses on the evaluation of alpha-amylase and alpha-glucosidase inhibition assays. The fifth chapter deals with a comparative study on the antidiabetic activity of ethyl acetate and aqueous leaf extracts of *S. kanarense*. The conceptual framework of the methodology is shown in Figure-1.

CHAPTER-2: PHYTOCHEMICALS SCREENING, ANTIOXIDANT ACTIVITY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

2.1 Introduction

This section represents the preface of the thesis, which is important for our research. Due to their importance in traditional medicine, plants have been considered a source of therapeutic agents for many years and are used in various diseases (Sharma *et al.,* 2009). Plants are a rich source of phytochemicals composed of phenols, terpenoids, tannins, steroids and saponins that have important biological and therapeutic effects, including antioxidant, antidiabetic, and antilipidemic activities (Rajasree *et al.,* 2016). The capacity of a plant to neutralize free radicals and reduce oxidative stress is related to its antioxidant capacity. Research findings from many clinical, experimental and epidemiological investigations show that the application of these antioxidants is

advantageous in the treatment of diabetes and its complications. According to many studies, DM is associated with increased formation of free radicals and a decrease in antioxidant potential (Matough *et al.*, 2012; Pratama *et al.*, 2022).

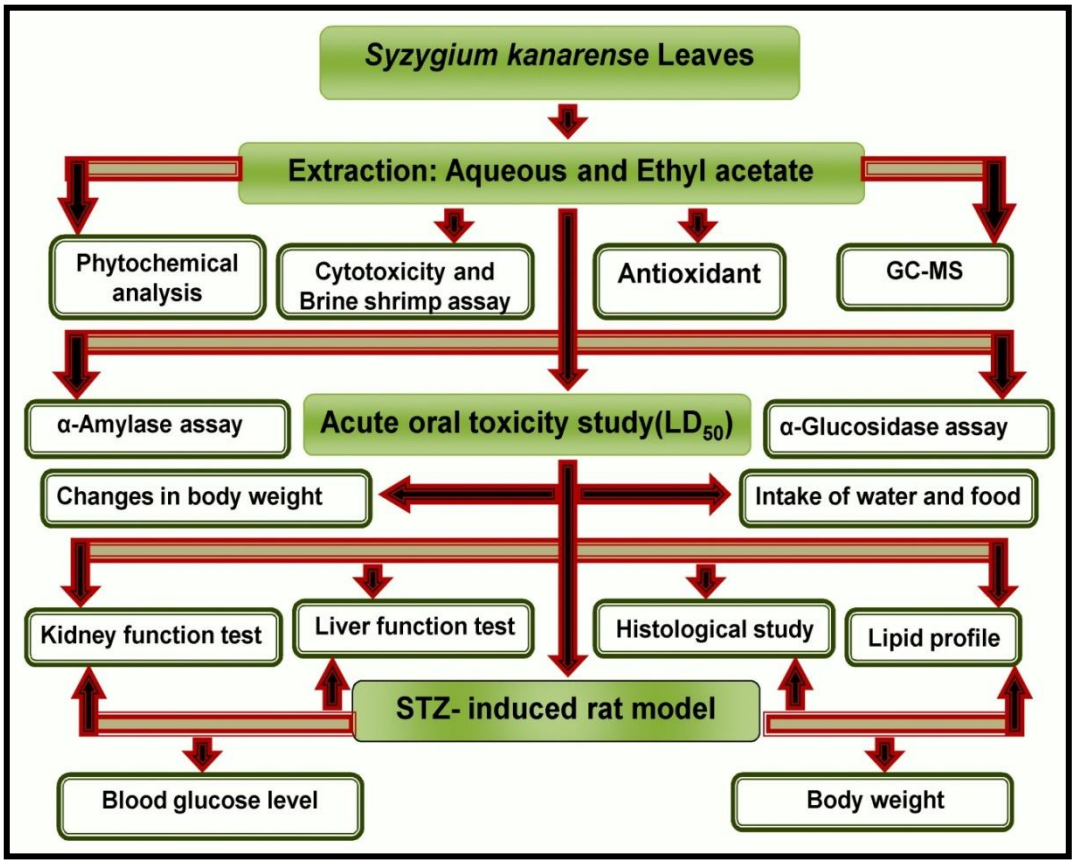


Figure 1: Flowchart of Methodology.

Medicinal plant extracts showed antioxidant and hypoglycemic properties. *Syzygium* species contain a high amount of antioxidants and show significant antidiabetic potential with less toxicity by controlling hyperglycemia. There are many reports that indicate that there is a close link between antioxidant activity and antidiabetic properties of medicinal plants (Ranilla *et al.*, 2010; Shori, 2015; Ouknin *et al.*, 2021). Therefore, a good herbal medicine with potential should have antioxidative properties. GC-MS relies on separating compounds based on the difference in their distribution coefficients (Hiller *et al.*, 2009). The Gas Chromatography (GC) portion separates compounds based on their volatility with flowing inert gas, which acts as a carrier in the stationary phase, and spectra of the compounds are identified by MS (Mass Spectrometer). MS identifies chemicals based on their mass to charge ratio (Hussain and Maqbool, 2014; Bhalla *et al.*, 2021). Therefore, this study aims at the preliminary phytochemical screening, quantification of phenols, antioxidant activity (DPPH and reducing power activity) and GCMS analysis of *S. kanarense* leaf extracts.

2.2 Materials and methods

This part covers the collection of plant material, preparation of plant extracts, preliminary qualitative and quantitative screening and detection of bioactive components by GCMS analysis, and *in vitro* antioxidant activity of *S. kanarense* leaf extracts.

2.2.1 Collection of plant material

The fresh mature leaves of *S. kanarense* were collected from Pilikula Nisargadhama, Mudushedde (N 12.9299, E 74.8958), Mangalore, Dakshina Kannada district, Karnataka, India (Voucher specimen NO. PND-800, flower: 2385 and plant fruiting: 2520). The plant materials were identified and authenticated by plant taxonomists, Prof. Krishna Kumar G, Mangalore University, Mangalagangothri and Dr. H. S. Shenoy, Principal Scientist, Pilikula Nisargadhama, Mudushedde, Mangalore. These specimens were deposited at the herbarium of the Pilikula Nisargadhama, Mudushedde, Mangalore, Karnataka.

2.2.2 Preparation of plant extracts

Plant leaves were washed in running tap water to remove dirt, followed by distilled water and extracts of different polarities by solvent, the leaf powder was extracted using the Soxhlet apparatus with petroleum ether, hexane, ethyl acetate and water according to standard technique (Nikhal *et al.,* 2010).

2.2.3 Preliminary screening of phytochemicals

The leaf extracts of *S. kanarense* were qualitatively examined for secondary metabolites such as phenols, alkaloids, saponins, tannins, terpenoids, and flavonoids (Edeoga *et al.*, 2005; Sawant and Godghate, 2013; Soni and Sosa, 2013; Yadav *et al.*, 2017).

2.2.4 Total phenolic content

The amounts of total phenolic content (TPC) present in the leaf crude extracts were estimated by Folin-ciocalteu method (Sheik and Chandrashekar, 2014). Gallic acid was used as the standard while TPC was expressed in mg/g Gallic Acid Equivalent (GAE).

2.2.5 Antioxidant assay

2.2.5.1 2, 2-diphenyl-1-picryl hydrazyl (DPPH) Radical Scavenging Assay

This DPPH method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity. The scavenging efficiency of free radicals of various crude extracts of *S. kanarense* leaves was estimated by following the protocol (Zhao *et al.*,

2014; Bhalla *et al.,* 2021). The values are expressed as IC_{50}. All samples were analyzed in triplicate.

2.2.5.2 Ferric reducing antioxidant power assay (FRAP)

A FRAP test is used to determine the overall antioxidant capacity of the extracts. For the crude leaf extracts of *S. kanarense*, a concentration of 20-100µg/mL was examined. The whole experiment was performed according to Benzie and Strain (1996). All samples were analyzed in triplicate.

2.2.6 Gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS analysis was performed to identify the volatile bioactive compounds present in various solvent extracts of *S. kanarense*. Identification was supported based on the molecular structure, molecular mass, and calculated fragments. Interpretation of GC-MS was conducted using the standard mass spectral database of Wiley and National Institute Standard and Technology (NIST) libraries. The relative percentage amount of every component was calculated by comparing its average peak area to the entire area (Prabhadevi *et al.,* 2012; Hussain and Maqbool, 2014).

2.3 Results

2.3.1 Phytochemical screening

The qualitative phytochemical screening revealed the presence of major classes of secondary metabolites such as phenols, tannins, terpenoids, saponins and steroids in ethyl acetate and aqueous extracts of *S. kanarense.* Tannins were absent in petroleum ether and hexane extracts. Alkaloids and flavonoids were not detected in any of the extracts.

2.3.2 *In vitro* antioxidant activity of *S. kanarense* leaf extracts

2.3.2.1 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

The maximum scavenging activity was observed in ethyl acetate and aqueous extracts, followed by petroleum ether and hexane extracts. The IC_{50} values of petroleum ether, hexane, ethyl acetate and aqueous extracts showed 26.24, 25.10, 12.38 and 14.17 µg/mL respectively, and compared with IC_{50} value of standard ascorbic acid.

2.3.2.2 Ferric reducing antioxidant power assay (FRAP)

Ethyl acetate and aqueous extract showed significantly greater FRAP action at all concentrations compared to petroleum ether and hexane extracts. All the extracts showed a dose-dependent inhibition as the concentration was increased.

2.3.3 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The chemical composition of *S. kanarense* leaf extracts was analyzed through GCMS. The GC-MS chromatogram of the ethyl acetate extract detected the Alloaromadendrene, Neophytadiene, Phytol, and gamma-sitosterol compounds. While, the aqueous extract showed the presence of 9-octadecenoic acid (z)-, methyl ester, Squalene, and Stigmast-5-en-3-ol, oleate compounds in the chromatogram.

2.4 Discussion

Phytochemicals were found to possess a wide range of effect that may support protecting against chronic disease (Fabricant and Farnsworth, 2001). The result of phytochemical screening of *S. kanarense* leaf extracts identified the presence of tannins, terpenoids, steroids, saponins and phenols. Some species of *Syzygium* exhibit hypoglycemic activities and may contain one or more active chemical compounds. Examples of chemical compounds from plants that have been shown potential to lower blood sugar levels are tannins, terpenoids and phenols (Miura *et al.*, 2005; Zwenger and Basu, 2008). The significant antidiabetic effect of ethyl acetate and aqueous crude extracts of *S. kanarense* may be attributed to the presence of the above-mentioned components, which may act synergistically to lower the blood glucose levels.

Diabetic patients were responsible for many factors related to the increase in oxidative stress. Among these factors, the most dominant is the auto-oxidation of glucose, which leads to the production of free radicals. Oxidative stress that occurs in diabetes has been directly linked to a reduction in antioxidant status. The antioxidant activity of plants has led to the development of drugs that offer protection and treatment against numerous diseases. Free radical scavengers may have an effect by lowering elevated serum glucose levels in diabetes and reducing secondary complications associated with diabetes. S. *kanarense* extracts have been studied for their potential antioxidant effects. The results of DPPH free radical scavenging capacity in *S. kanarense* extracts were expressed as percentage inhibition. The results suggest that there is no highly significant variation between the DPPH free radical scavenging capacity of the ethyl acetate and aqueous extracts of *S. kanarense* leaves.

Plants are the main source for the discovery of novel compounds with therapeutic value for drug development (Bnouham *et al.*, 2006; Wadkar *et al.*, 2008; Tundis *et al.*, 2010; Shori, 2015). In the present study, ethyl acetate and aqueous extracts of *S. kanarense* were subjected to GC-MS analysis. The GC-MS spectrum confirms the presence of

different bioactive compounds in ethyl acetate and aqueous extracts with different retention times. The gas chromatogram showed the presence of relative concentration of different compounds found in *S. kanarense* extracts getting eluted at different retention times. The phytocomponents were identified taylorione, hexadecanoic acid, methyl ester, methyl stearate, phytol in ethyl acetate extract and 9-octadecenoic acid (z)-methyl ester, stigmast-5-en-3-ol, oleate, and squalene in the aqueous extract. The identified compounds mentioned above are known to possess many biological activities such as antioxidant, antidiabetic, hypocholesterolemic activities and hepatoprotective activity. Therefore, the aqueous and ethyl acetate extracts of *S. kanarense* may act as a potential source of the antioxidant mechanism in diabetes and its complications.

2.5 Conclusion

The present investigations demonstrate that ethyl acetate and aqueous extracts of *S. kanarense* contain compounds with potential applicability for antidiabetic activities. In addition, the ethyl acetate and aqueous extracts showed potential bioactive components and were also significantly rich in antioxidant and phenols compared to petroleum ether and hexane extracts.

CHAPTER-3: TOXICITY STUDY ON *SYZYGIUM KANARENSE* LEAF EXTRACTS

3.1 Introduction

Plant-based natural products are a harbinger of so many useful and their derivatives have traditionally been a rich source of active ingredients in medicines. The utilization of plant-derived products has increased due to the fact that they do not induce any toxic effects (Yuan *et al.,* 2016). In ethnomedicinal, most medicinal plants play an important role in the treatment of multiple maladies or ailments with lesser or no side effects. There are many benefits of using plant-based natural products for maladies and health-related issues that can also be effectively reduce. However, traditional medicine remains the background for the improvement of new drugs (Patwardhan *et al.,* 2004). The increasing variety of herbal product users globally and the lack of historical proof on the safety report of herbal medicinal products are required to carry out toxicity studies (Saad *et al.,* 2006).

The research showed many medicinal plants and their components to investigate their toxicity potentials as they generate a wide range of drugs with therapeutic benefits (Patil *et al.,* 2010). The plant of the Myrtaceae family is rich in fruits, and volatile oils are normally

used as traditional medicine (Borah *et al.,* 2019). The chemical composition of the essential oil of *S. kanarense* from the aerial parts was investigated by Joshi *et al.* (2017). However, this is the first report on toxicological research from leaf extracts of *S. kanarense*. Therefore, an attempt was made to carry out the *in vitro* toxicity and acute oral toxicity experiment on *S. kanarense* leaf extracts using Wistar rats.

3.2 Materials and methods

3.2.1 Cytotoxicity by MTT assay on NIH/3T3 cell line

The MTT assay is a cytotoxicity test of metabolic competence based on the assessment of mitochondrial performance. It is a colorimetric assay based on the transformation of yellow tetrazolium bromide (MTT) to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells (Shoemaker *et al.,* 2004; Peng *et al.,* 2005). The effect of *S. kanarense* leaf extracts on cytotoxicity was determined according to the protocols of Mosmann (1983). The percentage of cell viability was determined.

3.2.2 Brine shrimp lethality assay

The toxicity of the leaf extracts of *S. kanarense* was evaluated by the brine shrimp lethality assay with slight modifications (Ogbole *et al.,* 2020). The method using *Artemia* (*Artemia salina*), is a simple toxicity assay for the natural product. The procedure determines lethal concentrations of extracts in a medium containing *Artemia*. The activities of a broad range of active compounds are manifested as toxicity to the shrimp.

3.2.3 Experimental animals approved by the animal ethical committee.

Animals were treated according to Animal Ethics Committee Guidelines and approved by the Institutional Animal Ethical Committee (IAEC) earlier to the beginning of the experiment, and laboratory animal care was taken according to CPCSEA regulations (Reg. no. BLDE/2019-20/645 and SCP/IAEC/F150/P165/2019).

3.2.4 Acute toxicity study

S. kanarense leaf extracts were evaluated for acute oral toxicity according to OECD Guidelines-425 (Saleem *et al.,* 2017) and are presented in Figure-2. The extracts were administered to a group of five female rats. Rats were observed for signs of toxicity. Then blood samples were collected by puncturing the heart under anesthetic conditions. Vital organs were excised and preserved in 10% formalin for histological studies. Later the LD_{50} value was calculated.

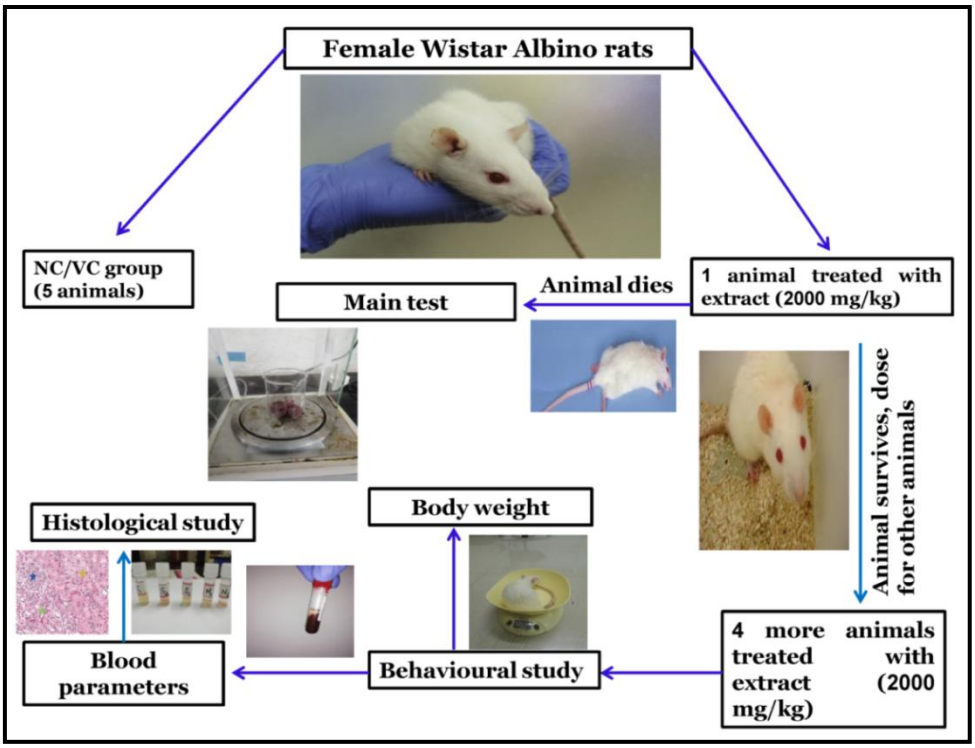


Figure-2: Schematic representation of acute oral toxicity.

3.3 Results

3.3.1 Cytotoxicity by MTT assay

Dose-dependent cell viability reduction after 24hr was observed in *S. kanarense* leaf extracts treated on NIH/3T3 cell line. The dose-dependent NIH/3T3 cell line viability was decreased after 24hr in the extracts-treated. The IC_{50} values of the cytotoxicity study showed in petroleum ether (>100µg/mL), hexane (>100µg/mL), ethyl acetate (425.07µg/mL) and aqueous (491.56µg/mL) extracts of *S. kanarense* leaves stimulated the growth of the NIH/3T3 cell line.

3.3.2 Brine shrimp lethality assay

The brine shrimp lethality tested in leaf extracts of *S. kanarense* of petroleum ether and hexane extracts appeared highly toxic with LC_{50} value less than 100 µg/mL. No toxic effect was reported in ethyl acetate and aqueous crude extracts with more than 70% shrimp survival at the highest concentration tested which was 1000 µg/mL.

3.3.3 Acute oral toxicity study on animal model

Based on the phytochemicals, antioxidant, *in vitro* toxicity study reports in all extracts, ethyl acetate and aqueous extracts were selected for acute oral toxicity study. According to OECD-425 guideline, acute oral toxicity results confirmed that the ethyl acetate and aqueous extracts of LD_{50} values were greater than 2000 mg/kg of body weight (B.W). No mortality was noticed in the oral acute toxicity study on Wistar albino rats.

3.4 Discussion

Medicinal herbs have been used cure a variety of ailments. Phytotherapy is gaining popularity as the WHO promotes appropriate ethnomedicinal usage and indicates the safety of herbal medications (Vaghasiya *et al.,* 2011). The FDA and WHO emphasizes the importance of conducting scientific studies to validate the efficacy and safety of herbal therapies (Setzer *et al.,* 2003). To ensure the safety of herbal drugs, preliminary toxicological testing is required. Acute oral toxicity is necessary to determine the safer dose range to manage the clinical signs and symptoms of the drugs (Saleem *et al*., 2017). The most important factor in this test is that the dose of the extract used for the test preparation for toxicity testing is the main parameter. The purpose toxicity test is to provide information on the adverse effects when the sample is administered to humans so that a safe dose can be determined. Therefore, the dose used must be equivalent to the general human dose. Other parameters observed were the assessment of the relationship between toxic effects and doses used in each group of animals, such as body weight changes, clinical symptoms, hematological parameters, clinical biochemistry, histology and other general effects or specific effects. No animal was found dead while some changes in behavioural patterns like sleep and itching were observed in both ethyl acetate and aqueous extracts treated groups in the first 24 hrs. Acute toxicity studies on ethyl acetate and aqueous extracts are practically non-toxic to Wistar rats. Statistically, no significant variations were found in rat treatment groups when compared with NC/VC group.

3.5 Conclusion

The ethyl acetate and aqueous leaf extracts of *S. kanarense* were shown to be non-toxic to female Wistar Albino rats as indicated by acute oral toxicity. Cytotoxicity studies by MTT assay on NIH/3T3 cell lines showed more cell viability in ethyl acetate and aqueous extracts compared to petroleum ether and hexane extracts. The cytotoxicity results depend on the high concentration of extracts showing more than 50% (IC_{50} value) cell viability in the MTT assay was selected for *in-vivo* toxicity. Based on these toxicity results, it is concluded that ethyl acetate and aqueous extracts (2000 mg/kg) were found to be non-toxic and can be used for further evaluation of the antidiabetic activity. A focus of the study in this direction certainly would bring awareness in availing the health benefits of natural plant extracts alternative to synthesized chemical toxicity.

CHAPTER-4: EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY ASSAYS

4.1 Introduction

Diabetes mellitus (DM) is characterized by a decline in cell membrane sensitivity, which is exacerbated by refined carbohydrate consumption, lack of exercise and obesity. In India, a high occurrence of the diabetes is noticed in the urban population (Misra *et al.,* 2001; Ramachandran *et al.,* 2004). The management of DM is recognized as global health issue and worldwide it attracted so much research attention to understand it further and development of successful treatment strategies. One of the emerging trends in the treatment of diabetes mellitus is research into the medicinal plant products for potent inhibitors of enzymes that metabolize carbohydrates. Inhibition of carbohydrate-digesting enzymes such as alpha-amylase and alpha-glucosidase in the gastrointestinal tract is a strategy for treating DM (Rasouli *et al.,* 2017; Sallau *et al.,* 2018). The alpha-amylase and alpha-glucosidase inhibitors slow the breakdown of carbohydrates in the small intestine, reduce postprandial blood glucose levels and have a beneficial effect on decreasing postprandial hyperglycemia levels. Several inhibitors of alpha-amylase and alpha-glucosidase have been isolated from medicinal plants to serve as an alternative to existing synthetic medications with higher potency and fewer side effects. Inhibitors of alpha-amylase and alpha-glucosidase are drug-design targets for the treatment of diabetes, obesity and hyperlipemia (Telagari and Hullatti, 2015).

The exploration of medicinal plant natural products for effective inhibitors of the carbohydrate metabolizing enzymes of alpha-amylase and alpha-glucosidase that would help regulate blood glucose levels is one of the growing topics in therapy for DM. Many parts of the plant, such as fruits, seeds, bark, and tea made from the leaves have been used in Asian countries to control diabetes. Therefore, the genus of *Syzygium* got recognition as plants with potential antidiabetic activity. The aim of this study on *S. kanarense* leaf extracts was discussed in detail in this chapter regarding their *in vitro* anti-diabetic through alpha-amylase and alpha-glucosidase inhibitor activities.

4.2 Materials and methods

4.2.1 *In vitro* antidiabetic activity of *S. kanarense* leaf extracts

The alpha-amylase and alpha-glucosidase inhibition assay were performed on *S. kanarense* leaf extracts and 50% inhibitory concentration (IC_{50}) was calculated.

4.2.1.1 Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay was carried out with a modified method by Nambirajan *et al.* (2018) and D'souza *et al.* (2021). The absorbance at 540 nm was used to calculate the percentage of alpha-amylase inhibitory activity. Acarbose was used as a standard control.

4.2.1.2 Alpha-glucosidase inhibitory assay

The alpha-glucosidase inhibition assay was performed using the methods of Chougale *et al*. (2009) and Nambirajan *et al.* (2018). The absorbance at 405 nm was measured with a spectrophotometer. Acarbose, the positive control was used in various quantities.

4.3 Results

4.3.1 *In vitro* study of alpha-amylase and alpha-glucosidase inhibition assay

Ethyl acetate and aqueous extracts of *S. kanarense* leaves were screened for their alpha-amylase and alpha-glucosidase inhibitory activity. The IC_{50} value of alpha-amylase in ethyl acetate extract was calculated to be 209.43µg/mL and in the aqueous extract to be 213.82µg/mL. Similarly, in the alpha-glucosidase inhibition assay, the IC_{50} values were found to be 212.15µg/mL in ethyl acetate extract and 247.46µg/mL in aqueous extract. Standard acarbose was used for both alpha-amylase and alpha-glucosidase inhibition assays. Both aqueous and ethyl acetate extracts showed dose-dependent inhibition when the concentration of alpha-amylase and alpha-glucosidase activity was increased.

4.4 Discussion

Maintaining blood glucose levels is an important process in controlling diabetes and its complications (Ratner, 2001; Dicarli *et al.,* 2003). The medical profession is still grappling with how to manage diabetes without having negative effects. It has been suggested that inhibition of alpha-amylase and alpha-glucosidase activity would delay carbohydrate breakdown and decrease glucose absorption, as leading to a reduction in postprandial blood glucose levels (Etsassala *et al.,* 2020). A therapeutic approach to control normal blood glucose levels is to suppress the production and/or absorption of glucose by inhibiting either alpha-amylase or alpha-glucosidase enzymes (Kunyanga *et al.,* 2011; Sudha *et al.,* 2011; Ponnusamy *et al.,* 2012). *S. kanarense* ethyl acetate and aqueous leaf extracts may inhibit alpha-amylase and alpha-glucosidase. The results obtained for alpha-amylase inhibition showed that *S. kanarense* has an IC_{50} value in both ethyl acetate

(209.43µg/mL) and aqueous (213.82µg/mL) extracts. This indicated that both the ethyl acetate and aqueous leaf extracts of *S. kanarense* inhibited alpha-amylase. The ethyl acetate extract was slightly more effective than the aqueous extract at a lower concentration. The inhibition of alpha-glucosidase in the aqueous extract showed less inhibition than in the ethyl acetate extract of *S. kanarense* leaves.

4.5 Conclusion

The *S. kanarense* leaf extracts showed a significant inhibitory effect against alpha-amylase and alpha-glucosidase activities. Inhibition of these enzymes by extracts could be useful in the treatment of postprandial hyperglycemia. It was found that the ethyl acetate and aqueous extracts of *S. kanarense* showed both anti-alpha-amylase activity and anti-alpha-glucosidase activities. However, the levels for anti-alpha-glucosidase activity were slightly lower than the anti-alpha-amylase activity.

CHAPTER-5: COMPARATIVE STUDY ON ANTIDIABETIC ACTIVITY OF ETHYL ACETATE AND AQUEOUS LEAF EXTRACTS OF *SYZYGIUM KANARENSE*

5.1 Introduction

Diabetes mellitus (DM) is a disease common in both developing and developed countries. It is characterized by hyperglycemia and modifications in carbohydrate, lipid and protein metabolism and relative deficiencies in insulin secretion and/or action and resulting in polyuria, polyphagia, ketosis, and weight loss (Vieira *et al.,* 2019). Type-1 diabetes accounts for 10% of all cases and it's characterized by a lack of insulin production, while type-2 diabetes accounts for more than 85% of all cases and is characterized by metabolic abnormalities such as insulin resistance and decreased pancreatic β-cell function (Jadhav and Puchchakayala, 2012). Diabetes is causing rapidly and the World Health Organization predicts that there will be 300 million or more diabetics by 2025 (Sy *et al.*, 2005; Zimmet *et al.*, 2015).

Herbal plants provide natural medicines that have fewer side effects and are more effective than synthetic alternatives (Batiha *et al.,* 2020). The World Health Organization encourages the evaluation traditional plants for diabetes treatment in view of their efficacy, non-toxicity and minimal side effects (Shokeen *et al.,* 2008). The research of phytoconstituents and toxicity study of *S. kanarense* leaf extracts were proved non-toxic in the experiment of *in-vitro* and *in-vivo* models. To the best of our knowledge, *S. kanarense*

is still under investigation and no research on *in-vivo* studies has been published. The current study investigated the antidiabetic activity of ethyl acetate and aqueous leaf extracts of *S. kanarense.* Both extracts yielded results that are comparable to that of a standard hypoglycemic medication drug, glibenclamide.

5.2 Materials and methods

5.2.1 Experimental design

All animals were housed under laboratory conditions and exposed to both food and water *ad libitum*. The animals were divided into non-diabetic rats (Group 1-2). The non-diabetic groups were treated in a similar way. Group-1 received no treatment; Group-2 was treated with the vehicle control (1% CMC). STZ-induced diabetic rats (Group 3-10) were used for long-term studies. The different groups within the STZ-induced rats were treated as follows: Group-3 received no treatment. Group - 4 and 5 received a low concentration of the leaf extracts (200 mg/kg B.W); Group-6 and 7 received leaf extracts at a concentration of 400 mg/kg; Group-8 and 9 received a higher concentration of the extracts (600 mg/kg b.w.); Group-7 was treated with the standard glibenclamide (10 mg/kg B.W).

Type-2 diabetes was induced in male Wistar rats weighing 200-300gm b.w. by intraperitoneal injection of streptozotocin (50 mg/kg) in 0.01 M citrate buffer at pH 4.5 (Musabayane *et al.,* 2005). About 4-5 mL of blood was collected from the experimental rats at the end of the experiment. Blood was centrifuged and serum was separated. Blood serum parameters were calculated calorimetrically using standard ready-to-use kits by AGD-2020 Clinical Chemistry Analyzer (AGD Biomedicals). The overall experimental design of *in vivo* antidiabetic study is shown in Figure-3.

5.2.2 Analysis of data

Results are reported as the mean ± standard error means (SEM). The SPSS program was used and analyzed the data. Statistical differences were evaluated by using one-way ANOVA (SPSS software and version-21), followed by the Dunnett-T3 test. Data were showed statistically significant at $p < 0.05$, moderate significant at $p < 0.01$, and highly significant at $p < 0.001$.

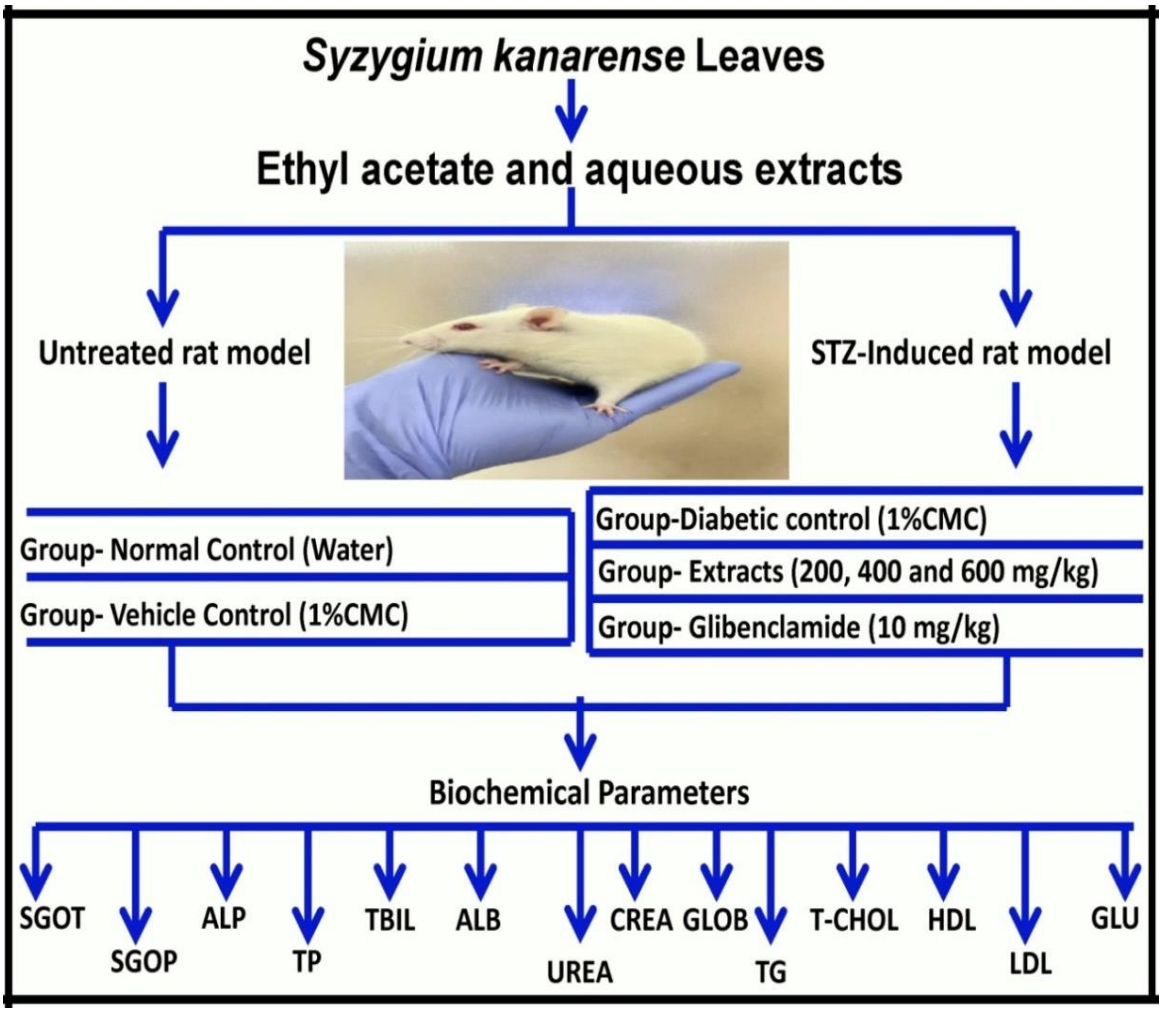


Figure 3: Experimental treatment design. [ALB – albumin, ALP – alkaline phosphatase, SGOT – serum glutamic-oxaloacetic transaminase, AST- aspartate aminotransferase, T-CHOL – total cholesterol, TG - triglyceride, HDL - high density lipoprotein, LDL - low density lipoprotein, CREA – creatinine, GLOB – globulin, TBIL – total bilirubin, TP – total protein, GLU – glucose].

5.3 Results

5.3.1 *In vivo* study of antidiabetic activity

Fasting blood glucose (FBG) levels ranged between 80 to 95 mg/dL in all the groups of rats prior to diabetic induction. Groups of diabetic rats treated with glibenclamide and extracts significantly ($p < 0.05$) reduced blood glucose levels compared to the untreated group of diabetic rats. STZ-induced (50 mg/kg, i.p) diabetic rats increased FBG level above 300 mg/dL after 72 hrs. Treatment with *S. kanarense* leaf extracts significantly reduced blood glucose levels.

5.3.1.1 Body weight (b.w.)

The body weight of the normal rats ranged from 200 to 230gm and increased to 240 to 260 gm at end of the experiment. On the other hand, the rats of the diabetic group

weighed 170 to 180gm on the 21st day of the experiment. Diabetic group rats treated with extracts showed a significantly ($p < 0.01$) increased in b.w. compared to rats in the diabetic group.

5.3.1.2 Effect of extracts on blood glucose level

STZ-induced diabetic rats treated with *S. kanarense* leaf extracts showed significant ($p < 0.001$) antihyperglycemic effects compared to diabetic group rats for up to 21 days. The groups of rats treated with both ethyl acetate, aqueous extracts (400and 600 mg/kg B.W) and the standard drug glibenclamide (10 mg/kg B.W) showed high significance ($p < 0.001$) compared to the rats in diabetic group. The ethyl acetate extract showed the highest activity in controlling blood glucose levels.

5.3.1.3 Blood serum analysis

After 21 days of the experiment, serum biomarkers such as serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were found to be significantly higher in diabetic group rats. In the groups of rats treated with extracts, the levels of SGOT and SGPT decreased significantly ($p < 0.001$ and $p < 0.01$) compared to diabetic group rats. Diabetic groups treated with aqueous and ethyl acetate extracts (400 and 600mg/kg B.W) and non-diabetic control groups were all within acceptable limits for urea and creatinine. Only the untreated group of diabetic rats showed significantly ($p < 0.001$) exceeding the upper limit for both urea and creatinine.

5.3.1.4 Histological study of pancreas, liver and kidney

The histological pancreas of the diabetic rat showed that the pancreatic cell size shrunk and the pancreatic islets being reduced. Cells in the NC (normal control) and VC (vehicle control) groups seemed to be normal. The histological examination of the kidney exhibited that the rats of non-diabetic group possess a normal glomerulus surrounded by Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes. The diabetic groups of rat kidney cells have abnormal glomerulus. Glibenclamide (Standard drug), ethyl acetate and aqueous extracts treated groups rats depicted the recovery of irregular glomeruli that were properly arranged.

5.4 Discussion

Results of the *in vivo* study demonstrated that the ethyl acetate, aqueous leaf extracts and reference drug (glibenclamide-10mg/kg) treated rats exhibited a significant time-dependent decrease in blood glucose levels on day 21 when compared to diabetic group rats. In this experiment, the ethyl acetate leaf extracts at dose of 600 mg/kg B.W

produced the highest antidiabetic effect and suggests that this may be an effective antidiabetic dose of *S. kanarense*. The variation between the highest and lowest doses may be due to the concentrations of phytochemicals and active compounds within the extracts. Diabetes induced by STZ has been associated with body weight loss due to increased muscle breakdown and tissue protein loss (Gupta *et al.*, 2012). The use of *S. kanarense* extracts of ethyl acetate and aqueous extracts (400 and 600 mg/kg B.W) improved the body weight in diabetic rats over the period of 21 days.

Biomarker enzymes such as alkaline phosphatase and alanine aminotransferase may be used for the evaluation of hepatic disorders (Ghandi *et al.*, 2012). A significant reduction of alkaline phosphatase in the extracts treated in diabetic group rats indicated a potential hepatoprotective role in preventing possible diabetic complications. From the histological analysis, the portal tracts in rats treated with the *S. kanarense* extracts appeared normal. The most significant change in the biochemical parameters measured and found in glucose levels. In all the STZ - induced diabetic rats glucose levels were elevated. According to statistical analysis, a highly significant ($p < 0.001$) difference was seen between all the diabetic group rats and the non-diabetic group rats. The treatment with the mid-dose (400 mg/kg B.W), high dose (6000 mg/kg B.W) and glibenclamide reduced the glucose levels as compared to the diabetic group of rats. The effect of leaf extracts (ethyl acetate and aqueous) treatment was concentration dependant, with better activity with the higher dose of extracts over the 21 day period that the experiment was carried out.

Natural supplements are becoming more popular in the treatment of diabetes as they are considered free from side effects and less expensive when compared to synthetic hypoglycemic agents, which have limitations such as being able to alleviate diabetic complications. However, it is important to note that the STZ-induced diabetic rats that were treated with the 600 mg/kg B.W dose of *S. kanarense* leaf extracts exhibited potential antidiabetic activity.

5.5 Conclusion

Diabetes was successfully induced by STZ in the animal model and used to assess the effect of *S. kanarense* leaf extracts on Wistar rats at doses of 200-600 mg/kg. The higher dose was associated with a decrease in blood glucose levels as well as significant changes in some of the key biochemical parameters in STZ-induced diabetic rats. According to the findings of this study *S. kanarense* leaf extracts has significant antidiabetic potential and may stimulate β-cells and protect pancreatic tissue.

SUMMARY AND CONCLUSION

This research work mainly deals with the qualitative, quantitative, antioxidant activity and GC-MS analysis of leaf extracts *S. kanarense*. Furthermore, toxicity study, alpha-amylase and alpha-glucosidase inhibition activity from leaf extracts of *S. kanarense*. Then, comparative study on the antidiabetic activity of ethyl acetate and aqueous leaf extracts of *S. kanarense* have been described in different chapters. The summary and conclusion for each chapter have been described below:

Summary

- The first chapter initially deals with the introduction and review of literature in detail diabetes mellitus and its applications, natural products, and their antidiabetic activity.
- Second chapter represents the importance of traditional medicine, a rich source of phytochemicals in plants and their therapeutic effects. GC-MS analyzed the bioactive compounds in leaf extracts of *S. kanarense* and may act as a potential source of the antioxidant mechanism in diabetes and its complications.
- Third chapter covers *in vitro* toxicity and acute oral toxicity of *S. kanarense* leaf extracts. The brine shrimps from petroleum ether and hexane extracts test showed highly toxic. In acute oral toxicity results confirmed that the ethyl acetate and aqueous extracts were non-toxic. So, the ethyl acetate and aqueous extracts for further study of antidiabetic activity on the animal model.
- Fourth chapter focuses on the ethyl acetate and aqueous leaf extracts of *S. kanarense* inhibited alpha-amylase. The inhibition of alpha-glucosidase in the aqueous extract showed less inhibition than in the ethyl acetate extract of *S. kanarense* leaves.
- Last chapter deals with the comparative study on the antidiabetic activity of the ethyl acetate and aqueous leaf extracts of *S. kanarense*. The results of this study showed that *S. kanarense* from ethyl acetate and aqueous extracts showed potential antidiabetic activity.

Conclusion

S. kanarense leaf extracts reported no toxicity effect on animal model. The aqueous and ethyl acetate extracts of *S. kanarense* were found to be anti-alpha-amylase activity and anti-alpha-glucosidase activity, although anti-alpha-glucosidase activity values were slightly lower than anti-alpha-amylase activity. Diabetes has been successfully induced by STZ in rats and used to determine the effect of *S. kanarense* leaf extracts at doses of 200-600 mg/kg. The higher dose reported a decrease in blood glucose levels and significant changes in some of the key biochemical parameters. The results of this study indicated that *S. kanarense* has significant antidiabetic potential and may act by stimulating of β-cells and protect pancreatic tissue. Further research works delineate a need to isolate and develop bioactive compounds derived from *S. kanarense* leaf extracts into antidiabetic agents and their effects should be validated *in vivo* and in clinical studies.

REFERENCES

American Diabetes Association. (2009). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 32,* 62-67.

Barrett, M. L., and Udani, J. K. (2011). A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): a review of clinical studies on weight loss and glycemic control. *Nutrition Journal, 10,* 1-10.

Batiha, G. E. S., Beshbishy, A. M., El-Mleeh, A., Abdel-Daim, M. M., and Devkota, H. P. (2020). Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of *Glycyrrhiza glabra* L.(Fabaceae). *Biomolecules, 10*, 352-370.

Benzie, I. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70-76.

Bhalla, N., Ingle, N., Patri, S. V., and Haranath, D. (2021). Phytochemical analysis of *Moringa oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications. *Saudi Journal of Biological Sciences, 28,* 6915-6928.

Bhat, M., Zinjarde, S. S., Bhargava, S. Y., Kumar, A. R., and Joshi, B. N. (2008). Antidiabetic Indian Plants: a Good Source of Potent Amylase Inhibitors. *Evidence-Based Complementary and Alternative Medicine, 1*, 324-328.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199-1200.

Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A., and Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity-a review of ten years of herbal medicine research (1990–2000). *International Journal of Diabetes and Metabolism, 14*, 1–25.

Borah, A., Pandey, S. K., Haldar, S., and Lal, M. (2019). Chemical composition of leaf essential oil of *Psidium guajava L*. from North East India. *Journal of Essential Oil Bearing Plants, 22*, 248-253.

Chaudhry, Z. R., Naseer, A., Chaudhry, S. R., Chaudhry, E. R., and Chaudhry, F. R. (2016). Comparison of extracts of *Syzygium aromaticum* on the weight of STZ-induced diabetic rats. *Journal of Islamic International Medical College, 11*, 24-28.

Chougale A. D., Ghadyale V. A., Panaskar S. N., and Arvindekar A. U. (2009). Alpha glucosidase inhibition by stem extract of *Tinospora cordifolia*. *Journal of Enzyme Inhibition and Medicinal Chemistry, 24*, 998–1001.

Colberg, S. R., Sigal, R. J., Fernhall, B., Regensteiner, J. G., Blissmer, B. J., Rubin, R. R., and Braun, B. (2010). Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes Care, 33*, 147-167.

Cukierman-yaffe, T., Gerstein, H. C., Williamson, H. D., Lazar, R. M., and Lovato, L., (2009). Relationship between baseline glycemic control and cognitive function in individuals with type-2 diabetes and other cardiovascular risk factors: the action to control cardiovascular risk in diabetes-memory in diabetes (ACCORD-MIND) trial. *Diabetes Care, 32***,** 221-226.

Di Carli, M. F., Janisse, J., Ager, J., and Grunberger, G. (2003). Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. *Journal of the American College of Cardiology*, *41*, 1387-1393.

D'Souza, J. N., Nagaraja G K., Meghana Navada, K., Kouser, S., and Manasa D. J. (2021). Insight into the impact of zinc doping on the structural, surface, and biological properties of magnasium oxide nanoparticles stabilized by *Vateria indica* (L.) fruit extract. *Ceramics International*, *47*, 29620–29630.

Donga, J. J., Surani, V. S., Sailor, G. U., Chauhan, S. P., and Seth, A. K. (2011). A systematic review on natural medicine used for therapy of diabetes mellitus of some Indian Medicinal Plants. *Pharma Science Monitor*, *2*, 36-72.

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, *4*, 685–688.

Etsassala, N. G., Badmus, J. A., Marnewick, J. L., Iwuoha, E. I., Nchu, F., and Hussein, A. A. (2020). Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Salvia aurita* constituents. *Antioxidants*, *9*, 1149.

Fabricant, D. S., and Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, *109*, 69-75.

Gandhi, G. R., Ignacimuthu, S., and Paulraj, M. G. (2012). Hypoglycemic and β-cells regenerative effects of *Aegle marmelos* (L.) Corr. bark extract in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology*, *50*, 1667-1674.

Gupta, R. K., Kumar, D., Chaudhary, A. K., Maithani, M., and Singh, R. (2012). Antidiabetic activity of *Passiflora incarnata* Linn. in streptozotocin-induced diabetes in mice. *Journal of Ethnopharmacology*, *139*, 801-806.

Hiller, K., Hangebrauk, J., Jager, C., Spura, J., Schreiber, K., and Schomburg, D. (2009). Metabolite Detector: comprehensive analysis tool for targeted and non-targeted GC/MS based metabolome analysis. *Analytical Chemistry*, *81*, 3429-3439.

Hussain, S. Z., and Maqbool, K. (2014). GC-MS: Principle, Technique and its application in Food Science. *International Journal of Current Science*, *13*, 116-126.

Jadhav, R., and Puchchakayala, G. (2012). Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. *Group*, *1*, 251-256.

Joshi, R. K., Sooryaprakash Shenoy, H., and Marati, R. (2017). Chemical composition of the essential oil of *Syzygium kanarense*: An endemic and rediscovered species from the Western Ghats, *Natural Product Communications, 12,* 1943–1944.

Konda, P. Y., Dasari, S., Konanki, S., and Nagarajan, P. (2019). *In vivo* antihyperglycemic, antihyperlipidemic, antioxidative stress and antioxidant potential activities of *Syzygium paniculatum* Gaertn. in Streptozotocin-induced diabetic rats. *Heliyon, 5,* 01373-01394.

Kunyanga, C. N., Imungi, J. K., Okoth, M., Momanyi, C., Biesalski, H. K., and Vadivel, V. (2011). Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. *Journal of Food Science, 76,* 560-567.

Lakshmi, V. J., and Manasa, K. (2021). Various Phytochemical Constituents and Their Potential Pharmacological Activities of Plants of the Genus *Syzygium*. *American Journal of Pharmatech Research, 11,* 68-85.

Li, W., Zhou, J., and Xu, Y. (2015). Study of the *in vitro* cytotoxicity testing of medical devices. *Biomedical Reports, 3,* 617-620.

Li, Y., Gao, F., Gao, F., Shan, F., Bian, J., and Zhao, C. (2009). Study on the interaction between 3 flavonoid compounds and α-amylase by fluorescence spectroscopy and enzymatic kinetics. *Journal of Food Science, 74,* 199-203.

Maahs, D. M., West, N. A., Lawrence, J. M., and Mayer-Davis, E. J. (2010). Epidemiology of type-1 diabetes. *Endocrinology and Metabolism Clinics, 39,* 481-497.

Matough, F. A., Budin, S. B., Hamid, Z. A., Alwahaibi, N., and Mohamed, J. (2012). The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos University Medical Journal, 12,* 5-18.

Misra, A., Pandey, R. M., Rama Devi, J., Sharma, R., Vikram, N. K., and Khanna, N. (2001). High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India. *International Journal of Obesity, 25,* 1722-1729.

Miura, T., Koike, T., and Ishida, T. (2005). Antidiabetic activity of green tea (*Thea sinensis* L.) in genetically type 2 diabetic mice. *Journal of Health Science, 51,* 708-710.

Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P., Aparna, S. R., Mangalapandi, P., and Samal, A. (2018). IMPPAT: a curated database of Indian medicinal plants, phytochemistry and therapeutics. *Scientific Reports, 8,* 1-17.

Musabayane, C. T., Mahlalela, N., Shode, F. O., and Ojewole, J. A. O. (2005). Effects of *Syzygium cordatum* (Hochst.)[Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology, 97,* 485-490.

Nambirajan, G., Karunanidhi, K., Ganesan, A., Rajendran, R., Kandasamy, R., Elangovan, A., and Thilagar, S. (2018). Evaluation of antidiabetic activity of bud and flower of *Avaram Senna* (*Cassia auriculata* L.) In high fat diet and streptozotocin-induced diabetic rats. *Biomedicine and Pharmacotherapy*, *108*, 1495-1506.

Newman, D. J., and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*, 770-803.

Nikhal, S. B., Dambe, P. A., Ghongade, D. B., and Goupale, D. C. (2010). Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. *International Journal of Pharmaceutical Sciences*, *2*, 30-32.

Ogbole, O. O., Ndabai, N. C., Akinleye, T. E., and Attah, A. F. (2020). Evaluation of peptide-rich root extracts of *Calliandria portoriscensis* (Jacq.) Benth (Mimosaceae) for *in vitro* antimicrobial activity and brine shrimp lethality. *BMC Complementary Medicine and Therapies*, *20*, 1-7.

Ouknin, M., Aghraz, A., Chibane, M., Boumezzourh, A., Costa, J., and Majidi, L. (2021). Enzyme inhibitory, antioxidant activity and phytochemical analysis of essential oil from cultivated *Rosmarinus officinalis*. *Journal of Food Measurement and Characterization*, *15*, 3782-3790.

Patel, D. K., Kumar, R., Laloo, D., and Hemalatha, S. (2012). Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine*, *2*, 411-420.

Patil, U. H., and Gaikwad, D. K. (2010). Phytochemical profile and antibacterial activity of stem bark of *Anogeissus latifolia*. *Pharmacognosy Journal*, *2*, 70-73.

Patwardhan, B., Vaidya, A. D., and Chorghade, M. (2004). Ayurveda and natural products drug discovery. *Current Science*, 25, 789-799.

Peng, L., Wang, B., and Ren, P. (2005). Reduction of MTT by flavonoids in the absence of cells. *Colloids and Surfaces B: Biointerfaces*, *45*, 108-111.

Ponnusamy, S., Zinjarde, S., Bhargava, S., Rajamohanan, P. R., and RaviKumar, A. (2012). Discovering Bisdemethoxycurcumin from *Curcuma longa* rhizome as a potent small molecule inhibitor of human pancreatic α-amylase, a target for type-2 diabetes. *Food Chemistry*, *135*, 2638-2642.

Prabhadevi, V., Sahaya, S. S., Johnson, M., Venkatramani, B., and Janakiraman, N. (2012). Phytochemical studies on *Allamanda cathartica* L. using GC–MS. *Asian Pacific Journal of Tropical Biomedicine*, *2*, 550-554.

Pratama, I. H., Girsang, E., and Suyono, T. (2022). Coriander Seed Extract (*Coriandum Sativum* L) As An Antioxidant. *International Journal of Health and Pharmaceutica*, *2*, 65-69.

Rajasree, R. S., Sibi, P. I., Francis, F., and William, H. (2016). Phytochemicals of Cucurbitaceae family—A review. *International Journal of Pharmacognosy and Phytochemical Research*, *8*, 113-123.

Ramachandran, A., Snehalatha, C., Baskar, A. D. S., Mary, S., Sathish Kumar, C. K., Selvam, S., and Vijay, V. (2004). Temporal changes in prevalence of diabetes and impaired glucose tolerance associated with lifestyle transition occurring in the rural population in India. *Diabetologia*, *47*, 860-865.

Ranilla, L. G., Kwon, Y. I., Apostolidis, E., and Shetty, K. (2010). Phenolic compounds, antioxidant activity and *in-vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*, *101*, 4676-4689.

Ratner, R. E. (2001). Controlling postprandial hyperglycemia. *The American Journal of Cardiology*, *88*, 26-31.

Rasouli, H., Hosseini-Ghazvini, S. M. B., Adibi, H., and Khodarahmi, R. (2017). Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food and Function*, *8*, 1942-1954.

Saad, B., Azaizeh, H., Abu-Hijleh, G., and Said, O. (2006). Safety of traditional Arab herbal medicine. *Evidence-Based Complementary and Alternative Medicine*, *3*, 433-439.

Saleem, U., Amin, S., Ahmad, B., Azeem, H., Anwar, F., and Mary, S. (2017). Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja Roxb*. roots in albino mice as per OECD-425 TG. *Toxicology Reports*, *4*, 580–585.

Sallau, A. B., Yakubu, R. N., Abdullahi, S., Salihu, A., and Boniface, B. Y. (2018). *In vitro* effect of terpenoids-rich extract of *Momordica charantia* on alpha glucosidase activity. *Vitae*, *25*, 148-153.

Sawant, R. S., and Gode, A. G. (2013). Qualitative phytochemical screening of rhizomes of *Curcuma longa* Linn. *International Journal of Science, Environment and Technology*, *2*, 634-641.

Setzer, R. W., and Kimmel, C. A. (2003). Use of NOAEL, benchmark dose, and other models for human risk assessment of hormonally active substances. *Pure and Applied Chemistry*, *75*, 2151-2158.

Sharma, R. A., Singh, B., Singh, D., and Ch, P. (2009). Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of *Boraginaceae* in India. *Journal of Medicinal Plants Research*, *3*, 1153-1175.

Sheik, S., and Chandrashekar, K. R. (2014). Antimicrobial and antioxidant activities of *Kingiodendron pinnatum* (DC.) Harms and *Humboldtia brunonis* Wallich: endemic

plants of the Western Ghats of India. *Journal of the National Science Foundation of Srilanka*, *42*, 307-313.

Shenoy, H. S., Krishnakumar, G. and Marati, R. (2015). Rediscovery of *Syzygium kanarense* (Talbot) Raizada (Myrtaceae) - an endemic species of the Western Ghats, India. *Journal of Threatened Taxa*, *7*, 6833–6835.

Shoemaker, M., and Cohen, I. and Campbell, M. (2004). Reduction of MTT by aqueous herbal extracts in the absence of cells. *Journal of Ethnopharmacology*, *93*, 381–384.

Shokeen, P., Anand, P., Murali, Y. K., and Tandon, V. (2008). Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. *Food and Chemical Toxicology*, *46*, 3458-3466.

Shori, A.B. (2015). Screening of antidiabetic and antioxidant activities of medicinal plants. *Journal of Internal Medicine*, *13*, 297–305.

Soni, A., and Sosa, S. (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy and Phytochemistry*, *2*, 22-29.

Sudha, P., Zinjarde, S. S., Bhargava, S. Y., and Kumar, A. R. (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complementary and Alternative Medicine*, *11*, 1-10.

Sy, G. Y., Cisse, A., Nongonierma, R. B., Sarr, M., Mbodj, N. A., and Faye, B. (2005). Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, *98*, 171-175.

Telagari, M., and Hullatti, K. (2015). *In-vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian Journal of Pharmacology*, *47*, 425-429.

Tiwari, P., Mishra, B. N., and Sangwan, N. S. (2014). Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *BioMed Research International*, *2014*, 1-18.

Tundis, R., Loizzo, M.R., and Menichini, F. (2010). Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini *Reviews in Medicinal Chemistry*, *10*, 315–331.

Vaghasiya, Y. K., Shukla, V. J., and Chanda, S. V. (2011). Acute oral toxicity study of *Pluchea arguta* boiss extract in mice. *Journal of Pharmacology and Toxicology*, *6*, 113-123.

Vieira, R., Souto, S. B., Sanchez-Lopez, E., Lopez Machado, A., Severino, P., Jose, S., and Souto, E. B. (2019). Sugar-lowering drugs for type-2 diabetes mellitus and metabolic

syndrome—strategies for *in vivo* administration: part-II. *Journal of Clinical Medicine*, *8*, 1332-1351.

Wadkar, K. A., Magdum, C. S., Patil, S. S., and Naikwade, N. S. (2008). Antidiabetic potential and Indian medicinal plants. *Journal of Herbal Medicine and Toxicology*, *2*, 45-50.

Xie, J. T., Wang, A., Mehendale, S., Wu, J., Aung, H. H., Dey, L., and Yuan, C. S. (2003). Anti-diabetic effects of *Gymnema yunnanense* extract. *Pharmacological Research*, *47*, 323-329.

Xu, H. (2010). Inhibition kinetics of flavonoids on yeast α-glucosidase merged with docking simulations. *Protein and Peptide Letters*, *17*, 1270-1279.

Yadav, R., Khare, R. K., and Singhal, A. (2017). Qualitative phytochemical screening of some selected medicinal plants of shivpuri district (mp). *International Journal of Life-Sciences Scientific Research*, *3*, 844-847.

Yuan, H., Ma, Q., Ye, L., and Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*, 559-577.

Zhao, Y., Du, S. K., Wang, H., and Cai, M. (2014). *In vitro* antioxidant activity of extracts from common legumes. *Food Chemistry*, *152*, 462-466.

Zimmet, P., Alberti, K. G., Magliano, D. J., and Bennett, P. H. (2016). Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nature Reviews Endocrinology*, *12*, 616-622.

Zwenger, S., and Basu, C. (2008). Plant terpenoids: applications and future potentials. *Biotechnology and Molecular Biology Reviews*, *3*, 1-7.

PUBLICATIONS

1. **Divakar, M. S.,** Raju K. C., Shivakumar H., and Karunakar H. (2020). Toxicological evaluation of ethyl acetate extract of *Syzygium kanarense* (Talbot) Raizada leaves in Wistar rats. ***Biomedical and Pharmacology Journal***, *13*, 497-509.

2. **Divakar, M. S.,** Raju K. C., Souvik D., Tapan K. C., and Sooryaprakash H. S. (2021).The evaluation of acute toxicity exposure to aqueous leaves extract of *Syzygium kanarense* (Talbot) Raizada. ***Journal of Microbiology, Biotechnology and Food Sciences***, *10*, 2219-2219.

3. **Divakar, M. S.,** and Raju K. C. (2021). Antidiabetic effects of unexplored *Syzygium kanarense* (Talbot) Raizada leaves extract in streptozotocin-induced diabetic rats. *An* ***International Journal of Phytomedicines and Related Industries***, *13*, 672-688.

CONFERENCES

1. Participated and presented orally on "*In-vitro* study of cytotoxicity and qualitative analysis of phytochemical in leaf extract of *Syzygium kanarense* (Talbot) Raizada" in ***International Conference on Medicinal Plants and Drug Disc*overy**" held on 18-20 July 2018, **Bangalore, India.**

2. Participated and presented orally on "Acute oral toxicity studies of a methanol extract of the leaves of *Syzygium kanarense* (Talbot) Raizada" in ***4th ICSTR Singapore – International Conference on Science and Technology Research,*** 15-16 November 2019, held at The **National University of Singapore Society (NUSS),** ***Organized by Scientific and Technical Research Association (STRA)***. **The Kent Ridge Guild House, 9 Kent Ridge Drive, Singapore.**

3. Participated and presented orally on "Evaluation of acute oral toxicity in ethyl acetate extract of *Syzygium kanarense* (Talbot) Raizada leaves in Wistar albino rats as per OECD 425" in ***International Conference on Plant Biodiversity and Environment Conservation (ICPBEC-2021),*** 20-21 May 2021, **Punjab Biodiversity Board. Punjab.**

Place: Mangalagangothri

Date: 24/08/2022

Signature of the Student

(Divakar M S)

Signature of the Research Guide